SEROTYPIC RECOMBINATION IN SALMONELLA

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Diagnosis of *Salmonella* serotypes is based largely upon the determination of thermostable somatic, (O), and flagellar thermolabile, (H) antigens, (1–3). These serological types have been codified within the Kauffmann-White scheme which is recognized by international agreement as the basis of a uniform classification. The increasing number of known serotypes has led to considerable speculation on the evolutionary mechanisms by which they are derived (4–7), particularly since the multiplicity of types is due in large part to numerous permutations of a set of components.

A technique for the genetic investigation of this recurrent pattern of combinations was provided by recent work (8) on genetic exchange in *Salmonella typhimurium*. It was found that genetic factors could be transferred individually from one strain to another by means of bacteriophage particles. The transfer of restricted fragments of hereditary material has been called "genetic transduction" to distinguish it from the more comprehensive sexual fertilization processes of other bacteria and higher forms. In *S. typhimurium*, certain bacteriophages appear to be able to function as the vectors of genetic transduction. The details of this mechanism are controvertible, but it may be imagined that disorganized fragments of nuclear material are released during the growth of a phage within the bacterial cell. Occasionally such fragments may be incorporated into maturing phage particles and be transported by them to cells of the second host bacterium. The successful implantation of the nuclear fragments in the organized

genetic structure of some of the new bacterial hosts, or their descendants, would complete the transduction.

The phages which function in *Salmonella* transduction grow in and lyse a considerable fraction of their host cells, so that high titer stocks can be prepared readily, but also leave sufficient bacterial survivors to be tested for transductive changes. These survivors are often but not always lysogenic for the phage, depending upon various cultural conditions and upon the adaptation of the phage to the particular host. The initial studies of transduction in *Salmonella* (8) were concerned primarily with nutritional, biochemical and drug-resistance factors, but established the rule that a given phage particle was likely to transduce no more than a single genetic factor. The frequency of successful transduction was so low (about one per million phages per trait) as to preclude coincidental transductions of two unrelated factors in any feasible experiment. This limitation did not necessarily reflect the assimilation of absolute genetic units into the phage particles, but may mean only that the nuclear fragments were, as a rule, too small to be likely to carry the determinants for any pair of bacterial traits that had been studied for correlated transduction.

In a preliminary test of inter-type transduction, Zinder and Lederberg (8) also showed that the *i* flagellar factor of *S. typhimurium* (IV V XII, *i:1,2*) could be transduced to *S. typhi* (IX XII, *d:—*) to produce a serotype previously unrecognized within the Kauffmann-White scheme, IX XII, *i:—*. In later experiments, including a wider variety of serotypes, three aspects of genetic determination of *Salmonella* flagella were distinguished: formation, locomotor function and antigenic specificity. In this study (9), concerned principally with non motile variants of *S. typhimurium*, at least seven distinct genetic factors or loci were implicated in the formation of flagella, two others in their locomotor function,

while the antigenic determinants formed a third, separate group. As a unique exception to the rule of non-correlation, one of the determinants for flagellar formation was found to be linked to a determinant for the H antigen, as will be discussed in further detail below. The present study is concerned with the exchange of flagellar antigenic factors among various *Salmonella* serotypes. A more detailed genetic analysis of flagellar determination and of phase variation is still in progress. The present report will emphasize experimental findings of particular interest to students of the serological diagnosis and antigenic structure of the *Salmonella* group.

MATERIALS AND METHODS

The phage used throughout these experiments was PLT22, derived from a lysogenic strain of *S. typhimurium* (8). This phage is adsorbed by most strains which carry the XII somatic antigen, excepting some strains of *S. paratyphi A* and *S. abortus-bovis*, but not so far as is known, by any others. Our experiments to date have, therefore, been confined to groups B and D, and some strains of group A. It is doubtful whether the receptor for the bacteriophage can be related to any specific fraction of the XII antigen, although the XII_2 component described by Kauffmann, (10), was previously suspected. Strains of *S. pullorum* recorded as XII_2 and XII_3 respectively have displayed an equal sensitivity to the phage, and transductions have been consummated to strains of *S. paratyphi A* whose lack of XII_2 has been confirmed by Dr. Kauffmann.

In order to function as a vector for the transduction of any character the phage must be grown on a source strain having the appropriate genetic quality. About half the types tested have proved to be suitable for the growth of the phage. Nonsensitive types are generally unsuitable as transductive donors, but will often function as recipients, if the phage is adsorbed. Such types are often lysogenic for a phage related to PLT22, or are poorly adapted to its growth and maintenance after the phage has been adsorbed.

Phage preparations were made as previously described (8, 9) by adding about .001 ml of a previous lysate to a freshly seeded broth culture of the bacterial host. After four to eight hours incubation at 37 C, during which clearing was sometimes but not always observed, the turbid cultures were pasteurized at 58 C for 40 minutes.

The bacteria were removed by centrifugation, and the supernates further sterilized and preserved with chloroform (11). Sterility tests were made routinely on 0.1 and 0.5 ml samples, and repeated when any result could be interpreted by the contamination of a lysate with surviving cells of the bacterial host. Entirely aside from the negative results of these controls, the transduction experiments usually resulted in types different from either parental strain, and often distinct from any types hitherto recorded, so that contamination can have played no significant role.

The low probability that any given phage particle will transduce a particular trait requires a selective technique for the isolation of new types. This was accomplished with the help of a modification (12) of the Wassèn-Gard method. Bacteria were inoculated into tubes or on plates of a semi-solid agar (9) to which appropriate anti-H serums were added to immobilize the recipient strain. The serum was generally added in such amounts that its final concentration was from 10 to 50 times the tube agglutination titer. The success of these experiments depends in large part on the use of serums sufficiently free from interfering antibodies which may inhibit the migration of the phases being sought. In most of the experiments recorded here 13 x 120 mm tubes of serum agar were used. These can be used immediately after being made up and can be incubated for several days or weeks without drying out. On the other hand, only one swarm can be selected per tube, while, with care, as many as 10 or 20 can be isolated from a small agar plate. However, plates must be dried carefully before they are used; surface spreading may interfere with the detection of sluggish swarms and, without special precautions, the plates will become desiccated within a few days.

In the presence of antiserum for the recipient strain, a new flagellar type was indicated by the outgrowth of a spreading swarm, usually within 24 to 48 hours. In the tube experiments, the swarms were permitted to spread to the bottom and isolated by melting off the column of agar. (This delicate operation is simplified if the tube is rotated rapidly while it is carefully heated over a small flame. The agar column will often break at a point which permits the unwanted overlying portion to be ejected intact. If this fails, a long wire is used to break the agar.) The

isolated outgrowths were streaked out on nutrient agar, and single colonies subjected to diagnostic tests. Further repeated single colony isolations were made if the results were at all ambiguous. For these qualitative experiments, the transducing phage was applied simply by suspending the recipient bacterium, from broth or agar culture, in the phage preparation, and inoculating about 0.1 ml of suspension per tube or plate.

Each culture was typed by the published methods practiced at the Communicable Disease Center (13, 14). Agglutinin absorptions were carried out as indicated, following the same practice. Most of the serums employed were prepared either at the Communicable Disease Center Laboratory at Chamblee, or at the Standards Laboratory, Central Public Health Laboratory, London, England. (The senior author is indebted to Dr. C. C. Spicer for furnishing the latter.)

Most of the *Salmonella* strains were taken from the stock culture collection (13), and were numbered as CDC-15, CDC-18, etc. Others will be documented as they are mentioned.

Abbreviations. TM = *S. typhimurium. TM2* = *S. typhimurium*, Lilleengen's strain 85, phage type 2 (8, 15). *PB* = *S. paratyphi B*. var. or strain as indicated. "ph1" and "ph2" = phase 1 and phase 2, respectively. Diagnostic formulae are given according to the Kauffmann-White scheme, e.g., IV V XII $i:1,2$ for diphasic *TM* (indicating somatic antigen IV V XII and flagellar antigens i (ph1) and $1,2$ (ph2)); IX XII $d:$— for the monophasic *S. typhi*. It should be understood that these formulae do not state all serological relationships, especially for the ph2 antigens which are customarily expressed "$1,2...$", "$1,5...$" etc.

The operation of a transduction experiment is symbolized A —x B gave C, or B x— A gave C, both of which may be read "phage grown on strain A was applied to strain B to give C" or "a factor from strain A was transduced to strain B to give C."

EXPERIMENTAL RESULTS

As a rule, the characteristic H antigen is not fully manifested in a recently purified *Salmonella* culture. For example, an individual colony of TM may agglutinate only in i, and not in $1,2$ serum. Subcultures will usually perpetuate the i response, but an occasional colony may no longer

react with i, but with $1,2$ instead. The i and $1,2$ responses are classified as ph1 and ph2 respectively. Cultures in ph2 likewise will give occasional subcultures which have reverted to ph1. Phase variation has some properties of spontaneous mutation (17), but may be regarded for the present, as the alternate expression of alternative antigenic potentialities inherent in a given serotype. When both phases are not observed in the course of ordinary cultivation, selection by antiserum against the overt phase may be applied. Cultures are described as monophasic when only a single flagellar phase is detectable, either in ordinary culture or by inoculation into semisolid agar with the appropriate serum (18).

Transductions to monophasic strains. The experimental results are classified according to the recipient strain. Most of the experiments with monophasic recipients concern *S. typhi* H901 and *PB* SW-666 and are recorded in Table I. H901 is a well-known strain used in serodiagnosis of typhoid fever (19). SW-666 is a galactose-negative fermentation mutant isolated (for methods, see 20) from a culture received from Dr. F. Kauffmann as #248, O-form from a tartrate-negative, monophasic *PB* (IV V XII $b:$—). SW-666 lends itself to serotypic transduction experiments, for, as shown previously (9), when motility is transduced to it from another type, this sometimes results in $b:$— flagella, but sometimes in flagella whose H antigen is characteristic of the donor strain. In this respect, SW-666 is atypical, as most O-forms will reveal only their own latent H antigen. The transductions to SW-666 listed in Table I were usually obtained by examining individual swarms on semisolid agar plates. Most of the swarms were of type $b:$—, and were discarded. Others were of the donor type. In some experiments, b antiserum was added to the plates to facilitate the isolation of donor type swarms. Correspondingly, the transductions to *S. typhi* H901 were carried out in the presence of d antiserum in tubes of semisolid agar. "Artificial phases" (21, 22) reacting $z_{33}:$— and $j:$— from SW-666 and H901, respectively, were noted in some experiments, regardless of the presence or absence of transducing phage, and are not recorded here.

It should be noted that SW-553 is one of a group of similar cultures received at the Communicable Disease Center (1520–51) from an

TABLE I

Transductions of flagellar antigens to monophasic strains, S. typhi H-901 and S. paratyphi B, SW-666

DONOR STRAIN				TRANSDUCTION TO S. TYPHI			TRANSDUCTION TO SW-666		
original recipient strains				IX XII	d:—	H-901	IV V XII	[b]¹:—	SW-666
S. abortus-equi	IV XII	[a]¹:enx	CDC-26				IV V XII	a:—	SW-985
S. sendai	IX XII	a:1,5	CDC-71	IX XII	a:—	SW-1040	IV V VII	a:—	SW-940
S. abony	IV V XII	b:enx	CDC-103	IX XII	b:—	SW-670	IV V XII	b:—	SW-677
S. altendorf	IV XII	c:1,7	CDC-125	IX XII	c:—	SW-902			
S. zega	IX XII	d:z_6	CDC-317				IV V XII	d:—	SW-987
S. san diego	IV XII	eh:enz_{15}	CDC-18	IX XII	eh:—	SW-668	IV V XII	eh:—	SW-664
S. enteritidis	IX XII	gm:—	CDC-64				IV V XII	gm:—	SW-679
S. dublin (0)	IX XII	[gp]¹:—	SW-553	IX XII	gp:—	SW-667	IV V XII	gp:—	SW-662
S. typhimurium	IV V XII	i:1,2	TM2	IX XII	i:—	SW-570	IV V XII	i:—	SW-623
S. javiana	IX XII	lz_{28}:1,5	CDC 732-49				IV V XII	lz_{28}:—	SW-984
S. heidelberg	IV V XII	r:1,2	CDC-16	IX XII	r:—	SW-687	IV V XII	r:—	SW-683
S. paratyphi B java ph 2	IV V XII	1,2:—	CDC-157	IX XII	1,2:—	SW-930	IV V XII	1,2:—	SW-901
S. paratyphi B	IV V XII	[b]¹:—	SW-666	IX XII	b:—	SW-1039			
S. gallinarum ?	IX XII	[gm]²:—	SW-970				IV V XII	gm:—	SW-982

¹ The antigen in [ ] is not overtly expressed, but has been inferred from other experiments.

² This antigen is not directly demonstrable, but is inferred from the present result.

outbreak among calves and pigs in Guatemala. It was suspected to be *S. dublin* at that time from its source and its biochemical behavior, but its flagellar antigens could not be typed, as SW-553 is stably nonmotile. However, the transduction of motility to SW-553 by phage grown on a variety of types has revealed the *gp* flagellar antigen, in agreement with the results of SW-553 —x SW-666, and SW-553 —x *S. typhi*, as recorded in Table I.

SW-970 was received from Dr. T. M. Floyd via Cmdr. L. A. Barnes and Dr. F. Kauffmann, and was originally isolated at Cairo from fresh chicken eggs. It had been typed as group D, nonmotile, and tentatively characterized as an aberrant (aerogenic) form of *S. gallinarum*. Attempts to transduce motility to it have failed (possibly because two or more distinct factors

S. pullorum CDC-75 and *S. gallinarum* CDC-74 —x *S. typhi* H-901 (in presence of *d*-antiserum) were inconclusive, as only *j* phases were secured. However, *S. gallinarum* CDC-74 —x SW-1040 (IX XII *a:—*, see Table I) has consistently engendered a type (SW-1041) IX XII *gm:—*, whose H-antigen, like that of transductions from SW-970, resembles that of *S. enteritidis*. Several other strains of *S. gallinarum* have behaved like CDC-74; experiments with *S. pullorum* strains have been inconclusive. A more detailed serological analysis of the uncovered H antigens is in progress.

The use of genetic transduction to reveal an otherwise suppressed antigenic potentiality is also exemplified by *S. abortus-equi* CDC-26 —x SW-666. CDC-26 has so far failed to engender any ph1 variants in selection in *enx* antiserum

TABLE II

Transductions of flagellar antigens to monophasic strains

DONOR				RECIPIENT[1]			RESULT[1]	
S. abony	CDC-103	IV V XII	b:enx	PB CDC-157		1,2:—	SW-937	b:—
							SW-926	1,2:enx
TM2		IV V XII	i:1,2	SW-926		1,2:enx	SW-933	i:enx
S. sendai	CDC-71	IX XII	a:1,5	SW-933		i:enx	SW-975	a:enx
TM2		IV V XII	i:1,2	PB CDC-157		1,2:—	SW-934	i:—
S. abony	CDC-103	IV V XII	b:enx	PB ph2?	SW-959	—:1,2	SW-995	—:enx
S. zega	CDC-317	IX XII	d:z_6	PB ph2?	SW-959	—:1,2	SW-999	—:z_6
S. zega	CDC-317	IX XII	d:z_6	PB ph2?	SW-960	—:1,2	SW-978	d:1,2
S. abortus-equi	CDC-26	IV XII	(a):enx	PB ph2?	SW-960	—:1,2	SW-994	a:1,2

[1] All strains in this column had the somatic antigen IV V XII.

for flagellar formation are lacking, and only one is transducible at one time (9).) SW-970 evidently carries a *gm* determinant, from the result of SW-970 —x SW-666, shown in Table I. Until *S. gallinarum* was more thoroughly examined, however, it could not be asserted whether this finding contradicted the identification of SW-970 with this serotype.

Unlike SW-970, authentic strains of *S. gallinarum* and *S. pullorum* have been ineffective as sources for the transduction of motility to SW-666 and to several other nonmotile mutants. Dr. B. Stocker (9 and private communication) noticed, however, that phage grown on *S. gallinarum* would transduce motility to *S. typhi* O-901, suggesting the applicability of *S. typhi* as a recipient strain to test for any latent H antigen that might be concealed in *S. gallinarum*.

agar, but Edwards and Bruner (23) demonstrated the suppressed *a* phase in other strains of the same serotype.

Transductions to ph1 monophasic types, SW-666 and H901, have given rise only to monophasic types which display the typical "specific" or ph1 antigen of the donor, except for the *1,2* antigen from *PB* java ph2, CDC-157.

Experiments with other monophasic recipients have been more restricted and are summarized in Table II. The transductions to CDC-157 further reflect the ph1 homologies of its *1,2* antigen. In addition, the monophasic CDC-157 has given rise to the diphasic SW-926, *1,2:enx*, an anomalous form which has its origin in the peculiar behavior of the *1,2* antigen. Transductions to SW-959 (a monophasic ph2 strain which biochemically resembles *S. paratyphi B*,

received originally from the Veterans Administration Hospital, Hines, Ill.), have engendered only monophasic types, whereas SW-960 (a similar culture originally isolated in Berlin, and forwarded by Dr. Kauffmann) has given diphasic derivatives. The genetic basis of these apparent alterations from monophasic to diphasic habit (and others in *S. abortus-equi* not reported here) is still in question.

Transductions to diphasic strains. These experiments were carried out very much like those above with H901, with the inclusion in the semisolid agar of antiserum for both phases of the recipient strain. The most extensive series involved a strain of *S. miami*, CDC 6500-51, IX XII $a:1,5$, summarized in Table III. As

that, in general, the phase that has been transduced corresponds to that of the donor parent. In every experiment, transduction to a diphasic strain results in the substitution of one of the two phases at any one occasion.

These results are paralleled by a smaller series using *TM* and *S. abony* as recipients (table IV). SW-674 (*S. dublin* —x *TM*) has been represented as IV V XII $gp:1,2$, but it was noted that colonies in the $1,2$ phase consistently reacted in low dilutions with gp antiserum as well, without other evidence of admixture. This confusion of phases may, however, be a peculiarity of the *TM* strain (experiments in progress). *Salmonella* cultures carrying gm, gp, gst, mt and similar flagellar antigens that have been isolated from

TABLE III

Transductions to Salmonella miami (CDC 6500–51)

DONOR				RESULT IX XII:	
—x Recipient S. miami	IX XII	a:1,5			
S. paratyphi B java	N97	IV V XII	b:—	b:1,5	SW-1028
S. paratyphi B java ph 2	CDC-157	IV V XII	1,2:—	1,2:1,5	SW-973
S. abony	CDC-103	IV V XII	b:enx	a:enx	SW-1022
				b:1,5	SW-1038
S. altendorf	CDC-125	IV XII	c:1,7	c:1,5	SW-1012
S. zega	CDC-317	IX XII	$d:z_6$	d:1,5	SW-1013
S. dublin O	SW-553	IX XII	[gp]	gp:1,5	SW-1016
S. san diego	CDC-18	IV XII	$eh:enz_{15}$	eh:1,5	SW-1014
S. typhimurium	TM2	IV V XII	i:1,2	i:1,5	SW-1015
				a:1,2	SW-1019
S. javiana	CDC 732-49	IX XII	$lz_{28}:1,5$	$lz_{28}:1,5$	SW-1017
S. heidelberg	CDC-16	IV V XII	r:1,2	r:1,5	SW-1018

in all previous experiments, the somatic antigen of the recipient strain was unaltered by flagellar antigen transductions.

Most of these results speak for themselves. The anomalous behavior of CDC-157 is here repeated, and results in a combination, IX XII $1,2:1,5$, SW-973. Except for their serologic identity, the alternation of phases resembles that of typical phase variation. Passage of SW-973 through agar with single-factor 2 and single-factor 5 serums, gives phases which react almost exclusively with anti-5 and anti-2 respectively. Except where indicated, no attempt was made to control the phase of the donor types, and the recipient *S. miami* was purposely a mixture of the a and $1,5$ phases. It will be noted

natural sources have almost invariably been monophasic, so that second phases of such cultures are often no longer sought in routine diagnosis. However, the recent isolations (24, 25) of diphasic *S. neasden* (IX XII $gst:enx$) and *S. worcester* (I XIII XXIII $mt:enx$) support the expectation that other diphasic types resembling SW-674 (table IV) and SW-1016 (table III) may be isolated in future surveys.

Table IV also illustrates again the anomalous behavior of CDC-157, which —x *S. abony* engendered SW-938, IV V XII $1,2:enx$. The possibility of reiterated transduction is also shown by several examples. *TM* —x *S. abony* ($i:1,2$ —x $b:enx$) gave SW-932, $b:1,2$ by the transduction of $1,2$ from *TM*. In a second run,

TM —x SW-932 now transferred the *i* factor, giving SW-943, *i:1,2*. Likewise, *S. abony* —x *S. javiana* (IV V XII *b:enx* —x IX XII *lz₂₈:1,5*) gave SW-980, IX XII *lz₂₈:enx*. In turn, *TM* —x SW-980 (IV V XII *i:1,2* —x IX XII *lz₂₈:enx*) gave SW-990, IX XII *i:enx*. In an extension of table III, *S. altendorf*, IV XII *c:1,7* —x SW-1022, IX XII *a:enx* gave SW-1050, IX XII *c:enx*. Thus, in numerous trials (including many not cited here) the results of one transduction have been quite as amenable to further transductions as the original parents. This statement must be qualified only in so far as the establishment of lysogenicity for the phage in the recipient strain interferes with its use as a donor.

Agglutinin-absorptions. In the preceding experiments the antigenic complex of a given phase was always transduced as a unit, i.e.,

ter was as stable as it had been in the parental strain (8, 9). They indicated further that transduction in *Salmonella* comprised not only the transfer of a genetic determinant, but the replacement of its homologue in the recipient bacterium. These conclusions have been substantiated by the present study. The routine typing procedure is designed to reveal the full range of antigenic potentialities of a strain being tested. The transductions to monophasic strains (table I) furnish compelling evidence for the irreversible replacement of the recipient antigen. Other experiments with diphasic recipients, and with serial transductions, have offered equal testimony that the replaced factors can no longer be detected either in the new types nor in transductions from these in turn. They can, of course, be restored by a subsequent trans-

TABLE IV

Transductions to S. typhimurium and S. abony, and S. javiana

DONOR			—x	RECIPIENT				RESULT		
S. abony	CDC-103	IV V XII	b:enx	TM2	IV V XII	i:1,2		IV V XII	i:enx	SW-698
								IV V XII	b:1,2	SW-699
S. dublin (O)	SW-553	IX XII	[gp]	TM2				IV V XII	gp:1,2	SW-674
TM2	IV V XII	i:1,2		S. abony	IV V XII	b:enx		IV V XII	i:enx	SW-941
								IV V XII	b:1,2	SW-932
TM2				SW-932	IV V XII	b:1,2		IV V XII	i:1,2	SW-943
S. zega	CDC-317	IX XII	d:z₆	S. abony	IV V XII	b:enx		IV V XII	d:enx	SW-1024
CDC-157	IV V XII	—:1,2		S. abony	IV V XII	b:enx		IV V XII	1,2:enx	SW-938
S. abony	CDC-103	IV V XII	b:enx	S. javiana	IX XII	lz₂₈:1,5		IX XII	lz₂₈:enx	SW-980
TM2	IV V XII	i:1,2		SW-980	IX XII	lz₂₈:enx		IX XII	i:enx	SW-990

such complexes as *1,2*; *1,5*; *enx*; *eh*; *lz₂₈*; *gp*; *gm* showed no separation into component factors. This may be compared with similar findings from mammalian immunogenetics, where single genetic factors determine cellular antigens of comparable mosaic complexity (26). On the other hand, these results are in direct contrast to the changes obtained within a single antigenic complex after cultivation in agglutinating serum (7, 27). The identity of transduced phases was first established by routine agglutination tests. In numerous instances it was confirmed by agglutinin absorption which showed that the transduced phase was identical with the antigenic phase as it existed in the donor strain. The agglutinogenic properties of new strains likely to be especially useful as antigens for the production of diagnostic sera are being studied further.

Stability of transduced characters. Previous studies had shown that the transplanted charac-

duction. For example, as shown in table I, TM2 —x SW-666 generates the type IV V XII *i:—*, SW-623. The *b* factor of SW-666 has evidently been replaced permanently by *i*, and *b* cannot be demonstrated in SW-623, either directly, or in further transductions in which SW-623 serves as donor or as recipient. However, *i* can again be replaced by *b* by means of transduction, SW-666 *b:—* —x SW-623 *i:—*, as has been verified by several trials.

DISCUSSION

The present data are insufficient for a detailed genetic interpretation of antigenic determination in *Salmonella*, and this must be deferred to a more suitable time and place. It is impossible, however, to disregard the most obvious features of these results, especially that the flagellar antigens fall into two distinct classes, corresponding as a rule to what have been loosely

called "specific" or ph1, and "nonspecific" or ph2. The factors within each group appear to be mutually homologous, replacing one another in transduction experiments. The outstanding discrepancy concerns CDC-157. This strain, designated as IV V XII *1,2:*—, was isolated from *PB* var. *java* (Army Medical School #N25, IV V XII *b:*—) as a rare exception to its monophasic habit (18). The *1,2* antigen of CDC-157 is identical with that of typical ph2 diphasic *PB* strains, and has been used for the production of standard *1,2...* antiserum. However, despite the serological identity of CDC-157, the genetic behavior of its *1,2* factor distinguishes it sharply from the *1,2* factors of diphasic organisms as recorded in the tables. Instead, it appears to be homologous with the set of ph1 factors, thus allowing for the production of types showing *1,2:1,5* and *1,2:enx.*

The potential combinations of the two major somatic antigens (IV XII: IX XII) and the available flagellar antigens of the two phases, according to the homology rules tentatively established would permit the generation of from 200 to 500 distinctive serotypes within these somatic groups alone depending upon the significance attributed to smaller differences. About 75 types are currently recognized. Some dozen additional diphasic combinations are cited in this paper, which, if they had been isolated from a natural source, would doubtless have deserved a place in the Kauffmann-White scheme. However, while transduction provides a laboratory method for the generation of almost any serotype (within these somatic groups) that is likely to be found in nature, and on the other hand, new serotypes are constantly being uncovered, it is by no means certain what role transduction may play in *Salmonella* evolution. It must not be assumed that we have elucidated all of the mechanisms of genetic variation potentially capable of engendering new types; and, in the last analysis, ecological rather than genetic forces are preeminently responsible for the distribution of types actually found, that is, for determining which of the numerous possibilities presented by genetic variation will be successful competitors in nature. This argument presumably accounts for the regularity with which cultures from "typhoid fever" conform to the serotype IX XII, Vi, *d:*— despite the

possibility of antigenic variants which has been exemplified in Table I.

The bearing of the present findings on nomenclatural as well as taxonomic problems is likewise conjectural. It has never been doubted that *Salmonella* serotypes must have arisen, and probably continue to arise, by evolutionary processes. The possibility that this process may include antigenic recombination, at least in part, does not detract in the least from the epidemiological and diagnostic applications of the Kauffmann-White scheme, so long as these continue to have an empirical basis. The convention of assigning specific epithets to serological types must be adjudged according to its own practical advantages and disadvantages; the nomenclature in general use has not been proposed seriously as a taxonomic scheme, the substantiation of which would require far more detailed knowledge of the evolution of bacterial species than can now be claimed.

The major diagnostic antigens of many of the types synthesized in the transduction experiments are similar to those of previously named types: in Table I, note SW-679: (in relation to) *S. essen*; SW-668: *S. reading*, monophasic variant (6); in Table 2, SW-978: *S. stanley*; SW-994: *S. kisangani*; SW-995: *S. abortus-equi*; in Table III, SW-1013: *S. ndolo*; SW-1014: *S. eastbourne*; SW-1022: *S. loma linda*; in Table IV, SW-699: *S. paratyphi B*; SW-943: *S. typhimurium*. If, in the application of the Kauffmann-White scheme, only the listed major antigens were considered, the synthetic types would necessarily qualify as examples of the named serotypes. It is not expected, however, that the substitution of flagellar characters has any influence on other distinctive features, e.g., biochemical properties and minor serological differences, or on clinical or epidemiological behavior. If absolute reliance could be placed upon the serotype for the prediction of pathogenic behavior (cf. 28) the synthetic types could be regarded as artificial. In practice, however, this reliance must be qualified, and this is perhaps another indication of a reshuffling of traits under natural conditions.

The most immediate practical contributions of this study are probably in the availability of monophasic types for use as antigens for serum production. However, monophasic strains are already available (12) for most of the antigens that can be transduced by present techniques.

SW-999, IV V XII —$:z_6$ is a notable exception, and is being tested for this purpose. Although it would be desirable for certain purposes, it is not presently possible to transduce flagellar factors to *Salmonella* strains carrying rare somatic antigens.

SUMMARY

The technique of genetic transduction has been applied to the exchange of flagellar antigens among various serotypes of *Salmonella*, somatic groups B and D. Each transduction resulted in the substitution or transfer of a single antigenic phase, whether to or from a monophasic or diphasic recipient strain. The types generated include several serotypes previously discovered and named, and others which so far have no place in the Kauffmann-White scheme. However, the precise role of transduction in the natural evolution of serotypes must be settled by further studies.

In the present material, the transduction of a flagellar antigen was unaccompanied by any other alterations in antigenic structure or biochemical behavior, so far as studied. In a few instances, transduction revealed the character of antigenic determinants which were not directly expressed in the source strain. Thus, the presence of an *a* phase in *S. abortus-equi* (IV XII [a]:enx) was confirmed, while *S. gallinarum* proved to carry a determinant for the *gm* antigen, which is unexpressed in the absence of flagella. In no case were the component factors of the complex antigens of a single phase (viz. *1,2...*, *e,h...*, *g,p...*, *e,n,x...*, *1,5...*) separated in transduction, and such tests as were made indicated that the transduced antigen was identical with the phase of the source strain.

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